

First record of *Kuhlia sauvagii* Regan, 1913 (Perciformes) in Mayotte and Réunion islands, Western Indian Ocean

by

Pierre FEUTRY* (1, 2), Magalie CASTELIN (1), Henri GRONDIN (3),
Corinne CRUAUD (4), Arnaud COULOUX (4) & Philippe KEITH (1)

RÉSUMÉ. - Premier signalement de *Kuhlia sauvagii* Regan, 1913 (Perciformes) à Mayotte et La Réunion, océan Indien occidental.

Kuhlia sauvagii Regan, 1913, anciennement synonyme de l'espèce *K. rupestris*, a été récemment revalidée par une analyse morphologique et méristique. Dans notre étude, nous avons séquencé le gène mitochondrial ND4 de ces deux espèces et utilisé les résultats pour procéder à des identifications moléculaires parmi différents lots de *Kuhlia*. *Kuhlia sauvagii*, supposée endémique de Madagascar, est signalée ici pour la première fois dans les îles de Mayotte (Comores) et de la Réunion (Mascareignes), élargissant ainsi son aire de répartition à l'ouest de l'océan Indien. La méthode d'identification moléculaire et la dispersion de cette espèce pendant sa phase larvaire marine sont discutées.

Key words. - Kuhliidae - *Kuhlia sauvagii* - *Kuhlia rupestris* - Western Indian Ocean Distribution - Molecular identification.

Kuhlia is a genus including 12 nominal species, which occur in tropical and subtropical fresh, brackish and marine waters of the Indian and Pacific oceans (Randall and Randall, 2001). The diadromous species *Kuhlia sauvagii* Regan, 1913 has been recently resurrected from synonymy with the widespread species *Kuhlia rupestris* (Lacepède, 1802) (Loiselle and Stiassny, 2007). According to

these authors *K. sauvagii* is endemic to Madagascar (Fig. 1).

In July 2008, during a sampling campaign for a project on the biodiversity of amphihaline organisms in insular systems of the South West Indian Ocean (DIAMSOI), specimens of *K. sauvagii* and *K. rupestris* were caught in Madagascar. Between 2006 and 2009, comprehensive surveys on the freshwater fish fauna allowed the capture of Kuhliidae post-larvae and juveniles in the streams of Mayotte and Réunion islands, respectively.

Our study confirms the presence of two freshwater *Kuhlia* species in the streams of Madagascar (i.e. *K. rupestris* and *K. sauvagii*), and provides the first record of *K. sauvagii* in the streams of Mayotte (Comoros) and Réunion (Mascarenes) islands based on mitochondrial DNA identification.

MATERIALS AND METHODS

Tissue samples from four specimens of *K. sauvagii* and four specimens of *K. rupestris* were collected in Madagascar after morphological identification following (Loiselle and Stiassny, 2007). The *K. sauvagii* samples were deposited at the Muséum national d'Histoire naturelle (MNHN), France (registration code: MNHN-IC 2011-0179 for three specimens from Ankatoka River and MNHN-IC 2011-0180 for one specimen from Ankavia River). 73 Kuhliidae post-larvae and juveniles from Mayotte and 75 Kuhliidae post-larvae from Réunion islands were collected (Fig. 1). No morphological identification was carried out on these specimens as only *K. rupestris* was supposed to occur in the rivers of these islands. Non-lethal fin clips were fixed in ethanol for all specimens except for three juveniles from Mayotte, which were collected as whole specimens and deposited at the Muséum national d'Histoire naturelle (MNHN), France (registration code: MNHN-IC 2011-1390) for three specimens from Coconi River).

Total genomic DNA was extracted from fin clips, using a semi-automated ABI PRISM™ 6100 Nucleic Acid PrepStation (Applied Biosystem). We amplified an 845-bp region of the mtDNA ND4 gene with the primers H12293-LEU (TTG CAC CAA GAG TTT TTG GTT CCT AAG ACC) (Inoue *et al.*, 2001) and ND4 (CAC CTA TGA CTA CCA AAA GCT CAT GTA GAA GC) (Arévalo *et al.*, 1994). Thermal cycling conditions consisted of an initial denaturation at 94°C for 1 min 30 s followed by 35 cycles of 94°C for 5 s, 55°C for 30 s and 72°C for 30 s, with a final extension step of 72°C for 5 min. A sequencing analysis followed on an ABI3730XL

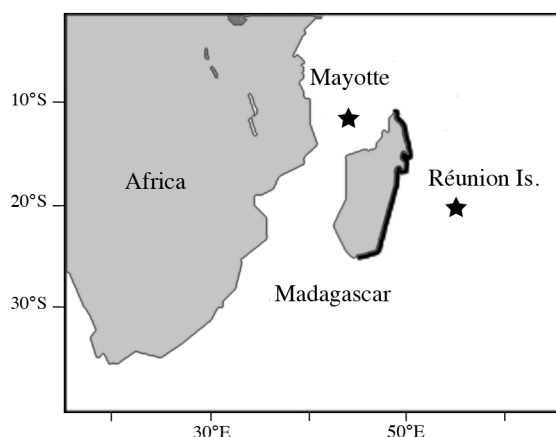


Figure 1. - New records for *Kuhlia sauvagii* shown with stars. Bold line shows the original geographical range of this species.

- (1) Muséum national d'Histoire naturelle, DMPA, Ichtyologie, UMR 7208, CP026, 57 rue Cuvier, 75231 Paris, France. [keith@mnhn.fr] [magcastelin@mnhn.fr]
- (2) Research Institute for Environment & Livelihoods, Charles Darwin University, Darwin, 0909 NT, Australia.
- (3) ARDA, route forestière de l'étang du Gol, 97427 Étang Salé, La Réunion. [grondin.arda@wanadoo.fr]
- (4) Genoscope, Centre national de séquençage, 2 rue Gaston Crémieux, CP5706, 91057 Evry cedex, France. [cruaud@genoscope.cns.fr] [acouloux@genoscope.cns.fr]

* Corresponding author [Pierre.Feutry@cdu.edu.au]

Table I. - Number (N), accession number (Acc. No) of the *Kuhlia* samples per species and per sampling location.

Species	Locality	River	N	Acc. No
<i>K. sauvagii</i>	Madagascar	Ankatoka	3	JQ413424-JQ413426
		Ankavia	1	JQ413425
<i>K. rupestris</i>	Madagascar	Ankatoka	4	JQ413420-JQ413423
	Mayotte	Coconi	3	JQ413417-JQ413419

sequencer (Applied Biosystems). Sequences were verified by forward and reverse comparisons before manual alignment.

RESULTS

Two haplotypes were found among the eight Kuhlidae from Madagascar, one corresponding to *K. rupestris* samples and the other to *K. sauvagii*. The sequences were deposited in Genbank (Tab. I). Forty-seven pairwise differences were observed between these haplotypes, corresponding to 5.56% nucleotide sequence divergence.

Among samples from Mayotte and Réunion Is., respectively one and eight individuals had sequences identical to *K. sauvagii* from Madagascar. The remaining samples from these islands (72 in Mayotte and 67 in Réunion Is.), including the three whole specimens, presented mitochondrial haplotypes that fell within the range of intraspecific divergence for *K. rupestris* (Feutry unpubl. data). The sequences of the three whole specimens from Mayotte, a posteriori identified as *K. rupestris*, both morphologically and molecularly, were deposited in Genbank (Tab. I). They differed by less than 1% with *K. rupestris* sequences from Madagascar samples.

DISCUSSION

Intraspecific divergences for the cytochrome c oxidase subunit 1 (COI) gene are rarely greater than 2%, even less when the species are sympatric (Avisé, 2000). ND4 was preferred to COI in this study as its evolution rate is slightly higher in the Kuhlidae (Feutry, unpubl. data). The 5.56% divergence between ND4 haplotypes from *Kuhlia* samples in Madagascar is more than twice the 2% threshold mentioned above and is in agreement with the presence of two Kuhlidae species in the fresh waters of Madagascar (Loiselle and Stiassny, 2007). The perfect match found between ND4 sequences of *K. sauvagii* from Madagascar and nine *Kuhlia* spp. from Mayotte and Réunion Is. identifies these individuals as *K. sauvagii*. This is the first record for this species in those islands. *K. sauvagii* and *K. rupestris* are similar species, both morphologically and ecologically. Only careful count of pored scales in the lateral line allows clear species determination. Adults (but not juveniles) can also be distinguished based on pigmentation patterning and coloration (Loiselle and Stiassny, 2007). Moreover, *K. sau-*

vagii individuals genetically identified in Mayotte and Réunion Is. represent less than 7% of all *Kuhlia* spp. sampled in this study. These reasons may explain why *K. sauvagii* has not previously been reported in these locations.

K. sauvagii is supposed to have an amphidromous life cycle (Loiselle and Stiassny, 2007), which means larvae of this species would spend some time at sea after hatching and presumably larval dispersal occurs during this marine phase. If so, the larvae would disperse along the coastlines of Comoros, Madagascar and Mascarenes Islands and recruit into adjacent freshwater habitats. All our samples from Mayotte and Réunion Is. were non-mature individuals, but since we discussed the results reported here with fish biologists from Réunion Is., they have identified one *K. sauvagii* adult based on morphological characters (Valade pers. com.).

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